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10 β ,17 α -Dihydroxyestra-1,4-dien-3-one: A bioprecursor prodrug preferentially producing 17 α -estradiol in the brain for targeted neurotherapy

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Abstract

Uterotrophic effect of 17 α -estradiol, the C17 epimer of the main human estrogen 17 β -estradiol, was shown to manifest in animal models at doses lower than those necessary for central outcome raising concerns about its potential to treat maladies of the central nervous system. We introduce here 10 β ,17 α -dihydroxyestra-1,4-dien-3-one (α -DHED) that acts as a bioprecursor prodrug producing 17 α -estradiol with remarkable selectivity to the brain and, therefore, without appreciable exposure of the periphery to the parent steroid. This distinguishing feature of α -DHED is shown by using an estrogen-responsive mouse model with complementary LC-MS/MS measurement of drug contents in target tissues. Our data warrant further research to fully establish the potential of α -DHED for a safe and efficacious 17 α -estradiol-based neurotherapy.

Keywords

17 α -estradiol; brain-selective bioprecursor prodrug; 10 β ,17 α -dihydroxyestra-1,4-dien-3-one; α -DHED; ovariectomized mice; uterotrophic effect; ERs binding; Porsolt swim test; LC-MS/MS

Estrogens have long been considered as potential central nervous system (CNS) drugs owing to a plethora of beneficial actions impacting the brain, including alleviating menopausal symptoms, affecting mood and protecting the organ against neurodegeneration.^{1,2} Any currently known non-invasive drug delivery method, however, produces systemic hormonal exposure upon chronic administration and, simultaneously, an increased risk for developing certain types of cancer and thrombosis, among other adverse events.^{3,4} The feminizing effect of estrogens has also been a serious drawback that greatly inhibits their practical drug

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[†]Author Contributions

K.P.-T. designed research, synthesized α -DHED, analyzed data and wrote the manuscript. V.N. conducted animal studies and run bioanalytical assays. L.P. designed research, conducted computational studies, analyzed data and co-wrote the manuscript. All authors have given approval to the final version of the manuscript.

K.P.-T. and L.P. are inventors in the patents covering the use of steroidal quinols as CNS-selective bioprecursor prodrugs and are co-founders of AgyPharma LLC with equity in the company that licensed the patents.

Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>: Characterization of α -DHED by LC-MS and GC-MS analyses

developments for gender-independent and safe treatments of centrally-regulated and estrogen-responsive maladies. Various approaches have been attempted to bypass these limitations; notably *via* the so-called chemical delivery system (CDS) to restrict estrogens' actions into the brain,⁵ and by the use of “non-feminizing” estrogens.^{6,7} While the CDS undoubtedly produces enhanced drug delivery into the brain compared to those of simple prodrugs such as estradiol benzoate, systemic hormonal exposure, inherent chemical instability and associated formulation problems make the otherwise interesting approach impractical.^{8,9} With “non-feminizing” estrogens on the other hand, the important genomic actions of the hormone associated with therapeutic benefits is lost,⁷ and these very lipophilic compounds mostly function as potent phenolic antioxidants.¹⁰

Due to its considerably decreased affinity to the nuclear estrogen receptors (ER α and ER β), the use of 17 α -estradiol (α E2), the C17 epimer of the main human estrogen 17 β -estradiol (E2), has been proposed instead of E2 to achieve a significantly diminished peripheral hormonal burden.^{11,12} Some reports have even suggested that α E2 as a CNS agent would completely eliminate undesired hormonal impacts on off-target estrogen-sensitive organs and, therefore, would offer a safe alternative to treat several estrogen-deficient brain and other CNS maladies.¹³ α E2 has also been found to be even more potent than E2, for example, in inducing CA1 pyramidal spine synapse density in ovariectomized (OVX) rats, suggesting potential role of α E2 in neuroprotection of the brain.¹⁴ Moreover, it has been proposed that α E2 is a viable candidate for the prevention/treatment of Alzheimer's disease owing to the involvement of ERs in the pathology of the disease.^{15,16} Similarly, α E2 efficaciously mitigated oxidative stress in another part of the CNS, namely in the retina, in a mouse model of oxygen-induced retinopathy.¹⁷ Altogether, these recent findings suggest that α E2 may have very important neural functions and translation potential in the treatment of various neurodegenerative disorders. However, α E2 also produces appreciable uterotrophic effect in OVX rodents, as we show here in agreement with others.^{18,19} Therefore, confinement of the action of this estrogen into the brain is highly desirable to avoid unwanted hormonal impact on the periphery. This is even more pressing in the context of translational research, as most estrogen-responsive maladies require long-term treatments that bring about critical consideration for safety and efficacy, highlighting thereby the need for brain-selective neurotherapy when proposing the use of α E2 as a preventative and/or curative agent.²⁰

We have recently reported a unique bioprecursor prodrug approach to target E2 into the brain without exposing the periphery to the hormone.^{21–24} The essence of this approach is that a facile bioconversion of the prodrug into E2 occurs only in the neuronal environment of the CNS. By the analogy with this approach, here we show that 10 β ,17 α -dihydroxyestra-1,4-dien-3-one (α -DHED, Figure 1 and Figure 2) also acts as a brain-targeting bioprecursor prodrug of α E2. The goal of the present exploratory study was to generate proof-of-concept data on the feasibility of α -DHED to act as the first brain-selective bioprecursor prodrug of α E2.

RESULTS AND DISCUSSION

The target compound α -DHED (Figure 1) was prepared by microwave-assisted stereoselective oxidation of the phenolic A-ring of α E2 using Pb(IV) acetate in glacial acetic acid analogously to a method reported before.²⁵ Compared to the parent drug, α -DHED (chemically a *para*-quinol) has significantly decreased lipophilicity (clogP 1.7 *versus* 4.0),²⁶ which should be advantageous in terms of brain uptake from the circulation. Therefore, it is expected that α -DHED produces a better “drug economy” and, thus, requires smaller dose to trigger the same neuropharmacological response as the direct administration of the highly lipophilic parent drug in animal models of estrogen-responsive and centrally regulated maladies. α -DHED is also anticipated to maintain the advantageous and experimentally confirmed drug-like properties of its β -stereoisomer that showed, among others, excellent oral bioavailability.²¹

Unlike α E2, the prodrug also has no appreciable affinities to the two nuclear estrogen receptors (ERs; ER α and ER β); in essence showing that α -DHED is indeed inactive as an estrogen. Table 1 shows the measured ER-binding affinities for the two isomeric estradiols, as well as for α -DHED when tested through binding assays based on time-resolved fluorescence resonance energy transfer (TR-FRET) employing ER α and ER β as target receptors, respectively.²⁷ The lack of α -DHED's binding to ERs is not surprising, as the presence of unshielded phenolic OH on the A-ring is an essential part of the pharmacophore in ER-affinity, and the prodrug does not possess this distinguishing functional group.¹⁰ Previously, we have also reported that the *para*-quinol scaffold of a steroid skeleton also resulted in the abolishment of binding affinity to other known steroid receptors.²¹

Through the use of the PM7 semi-empirical quantum chemical method,²⁸ a computer-aided mechanistic study on the bioprecursor prodrug's reductive bioactivation to α E2 in the brain confirmed a transition state ($G^\circ = 14.7$ kcal/mol, where G° denotes the change in Gibbs free energy under standard conditions; 1 atm pressure and 298 K temperature) leading to hydride (H^-) transfer from NADPH to C3 of the A-ring of α -DHED. Then, water elimination and subsequent re-aromatization of the A-ring affords α E2 having the phenolic A-ring. The overall process summarized in Figure 2 is spontaneous based on the calculated standard free energies ($G^\circ = -8.8$ kcal/mol).

The results summarized below demonstrate the preferential brain-selective formation of α E2 from α -DHED. We used an established animal model to probe the capability of the prodrug to produce α E2 in the brain and, consequently, to trigger antidepressant-like effect, without peripheral hormonal exposure. This neuropharmacodynamic response evoked by exogenously administered estrogens has been well-established,^{30,31} and the Porsolt swim test (PST, where the measured immobility time is related to a depressive state³²) offers a convenient paradigm for monitoring this effect. Accordingly, PST was used for the initial *in vivo* assessment of brain delivery of α E2 *via* its bioprecursor prodrug introduced here, and we indeed obtained statistically significant effect of treatments by one-way ANOVA both for α E2 ($F=8.52$, $P<0.001$) and α -DHED ($F=26.2$, $P<0.001$). Using OVX animals, peripheral exposure to circulating estrogens could also be easily surveyed by measuring wet uterine weight,^{7,8,21} and the response was particularly robust when experiments involving α E2

injections were evaluated statistically (one-way ANOVA: $F=82.3$, $P<0.001$). At the highest experimental doses employed, an antiestrogen (fulvestrant, a.k.a. ICI 182,780) was also co-administered with our test agents to confirm that ERs, or at least one of them,³¹ was responsible for the observed antidepressant-like and uterotrophic effects. Fulvestrant is the 7 α -alkylsulfinyl derivative of E2 that antagonizes ERs without agonist effect and is capable of crossing the BBB from the circulation.^{7,21,33} In addition, we used a validated qualitative assay to measure estrogen concentrations in target tissues by liquid chromatography–tandem mass spectrometry (LC–MS/MS).³⁴

A dose-dependent reduction in PST immobility was detected for the direct administration of the parent estrogen (α E2, Figure 3) and, expectedly, a dose-dependent effect was also observed upon administration of the prodrug (Figure 4). While s.c. administration of α -DHED at 30 μ g/kg body weight doses resulted in immobility times that were significantly decreased compared to those of the control group, statistically significant differences were obtained only at 100 μ g/kg s.c. doses for the direct administration of α E2. This has supported the notion about improved drug-like properties of α -DHED compared to the parent estrogen, including a better “drug economy” owing to the targeted drug delivery into the brain *via* the prodrug. The more robust behavioral response after α -DHED treatment compared to that of α E2 was also indicated by the displayed Cohen’s d values estimating effect size, as shown in Figures 3 and 4. It is noteworthy that E2 was significantly more potent than α E2 in PST using identical treatment regimen.^{21,35}

The antiestrogen fulvestrant completely reversed the antidepressant-like effect evoked by α E2 when it was co-administered either with the prodrug or with the parent drug, and the measured immobility times were not statistically different from that of vehicle-treated control group (Figure 3 and Figure 4). We also confirmed that fulvestrant did not exhibit intrinsic antidepressant-like effect (immobility times of 237 ± 10 s and 244 ± 33 s were observed for the fulvestrant- and vehicle control-treated groups, respectively).

Importantly, no statistically significant increase in wet uterine weights compared to those of control animals were observed in animals treated with α -DHED (Figure 4) at the dosing protocol utilized here based on our previously published studies.^{7,8,21,36} For example, the average wet uterus weight was 19 ± 3 mg even when animals received daily (q.d.) 300- μ g/kg body weight dose of prodrug for five consecutive days. In comparison, uterus wet weight was 17 ± 4 mg in the control group. In contrast, α E2 already produced a significant increase in uterus weight (46 ± 8 mg) even at 30 μ g/kg body weight dose that was insufficient to trigger antidepressant-like response (Figure 3). The observation of this characteristic uterotrophic effect involving fluid imbibition of the tissue due to exposure to estrogen²¹ strongly implies that α E2, albeit a much weaker estrogen compared to E2 (Table 1), indeed manifest significant peripheral hormonal burden in OVX animals.

Although uterotrophic effect, a very sensitive marker of circulating estrogens,²¹ could not be observed even at a high dose of α -DHED (300 μ g/kg body weight, Figure 4), we conducted confirmatory pilot LC-MS/MS measurements³¹ in serum and uterus, as well as in a representative brain region (cortex)²¹ after the conclusion of the behavioral studies.

We focused drug quantitation on the pharmacologically active α E2 with confirming the distribution of fulvestrant throughout the body including the brain, when applicable within the scope of the pilot study summarized in Table 2. We measured a high level of circulating α E2 (approximately 5 ng/mL) in animals treated with the parent compound only (at 300 μ g/kg body weight) and in those that also received the antiestrogen in addition to the test agent. Since there was no statistically significant difference among these treatment groups, Table 2 shows data pertaining to the administration of the test agents only. On the other hand, endogenous serum E2 levels in the control animals were less than 10 pg/mL (typically observed in OVX animals²¹) and, thus, we could not detect an increase in circulating E2 upon α E2 treatment, indicating lack of α E2's conversion to the endogenous 17 β -stereoisomer. Lowest level of quantitation in this assay for E2 was about 10 pg/mL.

Along with the high level of circulating α E2, the uteri of these animals showed a large concentration of α E2 (about 9 ng/g tissue, Table 2), independently whether fulvestrant was used or not. When fulvestrant was used at daily 4 mg/kg body weight s.c. dose, its concentrations in the serum and uterus were about 84 ng/mL and 150 ng/g, respectively (Table 2), and we did not find statistically significant changes in these measures when the antiestrogen was used alone or together with the test agents. However, the antiestrogen at the doses employed was capable of only partially reversing the profound uterotrophic effect by 300 μ g/kg body weight q.d. α E2 injections, as the wet uterus weight decreased from 89 ± 17 mg (α E2 only) to 29 ± 4 mg (α E2 + fulvestrant); yet the latter was still significantly different from that of control (20 ± 2 mg), as displayed in Figure 3. Interestingly, we also found a significant increase of E2 in the uterus of the α E2-treated animals (approximately 2 ng/g tissue *versus* 0.4 ng/g tissue for the control group) suggesting that locally synthesized E2 from α E2 may be responsible, at least in part,¹⁹ for the uterotrophic effect triggered by α E2. Further elucidation rigorously focusing on this observation will be addressed in a separate study.

In contrast, and very importantly, no α E2 or an increase in circulating endogenous E2 was found in the serum after α -DHED prodrug treatment (and, again, independently of fulvestrant co-administration)—consistent with the lack of uterotrophic effect (Figure 4). In addition, blood E2 level was not different from that of the control group (Table 2). Due to lack of circulating α E2 after the prodrug treatment, the uteri of the corresponding animals also did not contain α E2, and their endogenous uterine E2 level (approximately 370 pg/g tissue) was statistically not different from those of OVX control group.

Even though we could not detect circulating and uterine α E2 in the α -DHED-treated animals, we measured about 9 ng/g tissue of α E2 without an increase in endogenous E2 (approximately 280 pg/g tissue, which is about the same level as in the control group, see Table 2) in cortex samples, indicating that the observed antidepressant-like effect (Figure 3) was triggered by α E2 formed from α -DHED in the brain, and not due to its conversion to the more potent E2 in this experimental model.^{7,8,21,35} It was noteworthy that, in contrast with a previous report,¹¹ we could not detect α E2 in the brains of the control group and, therefore, could not confirm that α E2 is an endogenous estrogen in the rodents used in this study. At the same time, α E2-treated animals had significantly less α E2 in the analyzed brain part (approximately 1.8 ng/g tissue) than that of prodrug-exposed animals (Table 2),

again, indicating the more favorable “drug economy” with the prodrug. It is important to emphasize, that drug quantitation was done from tissues collected after anesthetized animals were perfused with cold saline through the left ventricle to remove avoid positive bias associated with measuring large blood drug concentration in the cortex and the uterus upon α E2 treatment.

In conclusion, the present study has shown that α -DHED efficiently converted to α E2 in the brain and, therefore, antidepressant-like effect was observed similarly to that of direct administration of the parent hormone. However, the observed central impact was not accompanied by detectable level of α E2 (or increase in endogenous E2) in the blood and, thus, α -DHED treatment was devoid of unwanted uterotherphic effect, a critical side-effect of any currently known non-invasive estrogen therapy. We have also generated promising data arguing for a better “drug economy” by the prodrug implicating therapeutic safety, as the target estrogen generated from α -DHED does not form and distribute in the periphery to trigger unwanted hormonal side-effects. An additional benefit of α -DHED would also be the isolation of central α E2 effect from the influence of circulating hormone, which could allow for a better understanding of this estradiol epimer’s central *versus* peripheral actions, as well as its contributions to the observed overall estrogenic effects in the body. Overall, the data presented here warrants further studies to realize the basic-science and translational potential of this bioprecursor prodrug.

EXPERIMENTAL SECTION

General.

α E2, reagents, analytical standards and solvents were purchased from commercial sources. For analytical and preparative thin layer chromatography aluminum oxide 60 F254 plates (MilliporeSigma) were used with hexane/ethyl acetate 1:1 (v/v) eluent. NMR spectra were recorded on a Bruker (Billerica, MA, USA) Fourier 300 HD instrument using tetramethylsilane as an internal reference and CDCl₃ as solvent. Chemical shifts are in ppm, and coupling constants are in Hz. High-resolution mass spectra ($M^+ - M > 40000$) were recorded on linear ion trap–Orbitrap (LTQ Velos Orbitrap Pro; Thermo Fisher Scientific, San Jose, CA, USA) hybrid mass spectrometer using an atmospheric pressure solids analysis probe (ASAP; M&M Mass Spec Consulting, Newark, DE, USA). An auxiliary flow of acetonitrile (0.05 mL/min) was supplied by the system’s built-in syringe pump promote the formation of protonated molecules during ionization. The instruments for liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS) were purchased from Thermo Fisher Scientific, San Jose, CA, USA. LC-MS was performed on a TQS Quantum Ultra system interfaced to a Surveyor MS Plus gradient LC pump and equipped with a Surveyor AS autosampler. GC-MS measurements were completed by using a Polaris Q instrument connected to a TRACE GC fitted with an AI 3000 autoinjector.

Synthesis of α -DHED.

The procedure followed our previously published protocol.²⁵ Briefly, α E2 (80 mg, 0.3 mmol, 1 eq) was dissolved in 5 mL of glacial acetic acid followed by the addition of 1.5 eq

(210 mg/95%, 0.45 mmol) of Pb(OAc)₄. Microwave irradiation (CEM Discover, Matthews, NC) in a 10-mL reaction vessel was applied for 15 min under temperature control set to 40 °C. The intermediate *para*-quinol acetate was not isolated, but hydrolyzed after removal of the solvent by evaporation followed by subsequent addition of 5 mL of NaOMe in MeOH (25% w/v) followed by microwave irradiation for 5 min. The solution was then poured on ice water and the precipitated crude product was collected. After drying, the product was purified by preparative TLC and isolated as a white solid with ~45% (~39 mg) overall yield. $R_f = 0.48$; HR-MS (ASAP) $[M+H]^+$ m/z 289.18014 ($\delta = 1.1$ ppm mass error compared to m/z 289.17982 calc. for C₁₈H₂₅O₃). Anal Calc. for C₁₈H₂₄O₃ × 0.25 H₂O: C, 73.82; H, 8.43; Found C, 73.72; H, 8.54. ¹H-NMR: δ 7.09 (d, J=10.5 Hz, H-1, 1 H), 6.18 (dd, J= 10.4 & 2.5 Hz, H-2, 1H), 6.00 (d, J = 2.4 Hz, H-4, 1H), 3.77 (m, H-17 β , 1H), 2.72 (td, J= 13.1 & 4.2 Hz, H-6, 2H), 2.26–1.10 (m, H-7, H-8, H-9, H-11, H-12, H-14, H-15, H-16, 13H), 0.76 (s, 18-Me, 3H).

Receptor Binding Assay.

Affinities to ER α and ER β receptors were characterized by SelectScreen™ Biochemical Nuclear Receptor Profiling Service by Thermo Fisher Scientific (Carlsbad, CA) using their LantaScreen™ TR-FRET competitive binding assay screening protocol.²⁷

Theoretical Calculations.

All models were constructed and visualized with the SCIGRESS suit (version FJ 2.8.1; Fujitsu–FQS, Poland) installed on a personal computer (PC) running under the Microsoft (Redmond, WA, USA) Windows 10 operating system. Structures for ground and transition states, as well as thermodynamic properties were calculated by using the PM7 and PM7-TS semi-empirical quantum chemical parameterizations²⁸ through the PC-version of the MOPAC2016 package.³⁷ The COSMO model²⁹ was employed to simulate continuum solvation in water (ϵ permittivity parameter set to 78.4).

Animals.

OVX young adult CD1 mice (30 ± 4 g body weight) were purchased from Harlan Laboratories (Indianapolis, IN, USA). All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of North Texas Health Science Center before the initiation of the studies. Mice were housed per cage in a room conditioned to 21–23°C with normal day/night cycles and they had *ad lib* access to food and water. Each animal was tested only once.

Porsolt Swim Test (PST).

Six or twelve animals per treatment group were used, similarly to earlier studies.^{7,8,36} Briefly, test agents were dissolved in corn oil. Fulvestrant was used at 4 mg/kg body weight³³ dose upon each scheduled administration. The control groups received vehicle only. Test compounds at various concentrations (Figure 2 and Figure 3) in corn oil vehicle were administered s.c. daily (q.d.) for five consecutive days. Antidepressant-like activity was evaluated 30 min after the last injection. The immobility time (s) was recorded for 6 min simultaneously by a trained observer who was blinded to the treatment in question.

LC–MS/MS assay.

E2 and α E2 analyses were done according to our published protocol.^{7,8,21,34} Briefly, the subjects were anesthetized after the behavioral test and blood was collected by cardiac puncture to obtain serum samples. The animals were then perfused with cold saline through the left ventricle^{8,21,33} to avoid bias associated with high blood content of α E2 (or fulvestrant, when applicable). Mice were then euthanized, target tissues were collected and processed immediately. Tissue homogenization (20% w/v in pH 7.4 phosphate buffer) was followed by the addition of d₂- α E2 and [¹³C₆]E2 internal standards. Liquid-liquid extraction was done with *tert*-butyl-methyl ether. After evaporation, the residue was dansylated for quantitation by the isotope-dilution principle.³⁴

Statistical Analysis.

Evaluations were done by one-way analysis of variance (ANOVA). Two-group comparisons employed *post hoc* Dunnett's tests. $P < 0.05$ was considered statistically significant. Effect sizes in the behavioral experiments were estimated as standardized differences between two means (Cohen's *d*) for the pairwise comparisons.³⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CDS	chemical delivery system
CNS	central nervous system
CND	E2, 17 β -estradiol
α-E2	17 α -estradiol
α-DHED	10 β ,17 α -dihydroxyestra-1,4-dien-3-one
ERs	nuclear estrogen receptors
G°	Gibbs free energy under standard conditions
Pb(OAc)₄	lead(IV) acetate
PST	Porsolt swim test
TR-FRET	time-resolved fluorescence resonance energy transfer

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Figure 1.

Synthesis of α -DHED. (i) $\text{Pb}(\text{OAc})_4$, glacial acetic acid, microwave (2.45 GHz, 0–300 W), 40 °C, 20 min; (ii) NaOMe in methanol, pH 9, microwave, 40 °C, 5 min. The intermediate (in bracket, R = Ac) was not isolated (i.e., the synthesis was a “one-pot” procedure).

Figure 2.

A mechanistic model for α -DHED's bioactivation to α E2. Semi-empirical quantum-chemical calculations (PM7 parametrization²⁸) were applied to the complete steroid structures and a mimic ($R = \text{CH}_3$) of $\text{NAD(P)H} \rightleftharpoons \text{NAD(P)}^+$ simulating solvation with water through the conductor-like screening model (COSMO).²⁹

Figure 3.

Immobility times in PST (open bars) and wet uterus weights (closed bars) after the s.c. administration of increasing doses of α E2 (30, 100 and 300 μ g/kg) or vehicle to OVX CD1 mice. Data are expressed as mean $\pm$ standard deviation (SD), $n=6$ except for the vehicle control group ($n=12$). Statistically significant difference from control (one-way ANOVA followed by *post hoc* Dunnett's tests) is denoted by asterisks; effect size of behavioral response is indicated by Cohen's d . The co-administration of the ER-antagonist fulvestrant (4 mg/kg body weight) completely reversed the behavioral response and partially reversed uterotherphic effect of 300 μ g/kg E2.

Figure 4. Immobility times in PST (open bars) and wet uterus weights (closed bars) after the s.c. administration of increasing doses of α -DHED (30, 100 and 300 μ g/kg) or vehicle to OVX CD1 mice. Data are expressed as mean $\pm$ SD, $n=6$, except for vehicle control group ($n=12$). Asterisk denotes statistically significant difference from control (one-way ANOVA followed by *post hoc* Dunnett's tests); effect size of behavioral response is indicated by Cohen's d . The antidepressant-like effect was completely reversible by co-treatment with the ER-antagonist fulvestrant (4 mg/kg body weight).

Table 1.

Summary of TR-FRET binding assays using ER α and ER β as target receptors.

Compound	IC ₅₀ (nM)	
	ER α	ER β
α -DHED	>1000	>1000
α E2	2.56	16.2
E2	0.33	0.41

IC₅₀: half maximal inhibitory concentration

Table 2.

Drug concentrations in blood, cortex and uterus after s.c. treatment of OVX CD1 mice (n=4) for 5 days q.d. with vehicle (control), the ER-antagonist fulvestrant (4 mg/kg body weight), α E2 (300 μ g/kg body weight) and α -DHED (300 μ g/kg body weight), respectively.

Test Agent	Serum (ng/mL $\pm$ SEM)			Cortex (ng/g $\pm$ SEM)			Uterus (ng/g $\pm$ SEM)		
	E2	α E2	Fulvestrant	E2	α E2	Fulvestrant	E2	α E2	Fulvestrant
Vehicle Control	<0.01	N.D.	—	0.21 $\pm$ 0.07	N.D.	—	0.44 $\pm$ 0.06	N.D.	—
Fulvestrant	<0.01	N.D.	84 $\pm$ 6	0.23 $\pm$ 0.04	N.D.	9 $\pm$ 2	0.38 $\pm$ 0.05	N.D.	150 $\pm$ 24
α E2	<0.01	5.4 $\pm$ 0.2	—	0.18 $\pm$ 0.02	1.8 $\pm$ 0.7	—	2.00 $\pm$ 0.45	9.0 $\pm$ 0.8	—
α -DHED	<0.01	<0.01	—	0.28 $\pm$ 0.09	8.9 $\pm$ 1.7	—	0.37 $\pm$ 0.10	N.D.	—

SEM is standard error; N.D. denotes not detected; long dashes indicate not applicable.